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Metal-Induced Inhibition of Glutathione S-Transferases

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METAL-INDUCED INHIBITION OF GLUTATHIONE S-TRANSFERASES

by

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The glutathione S-transferases comprise a group of multi-functional enzymes involved in the biotransformation/detoxication of a broad spectrum of hydrophobic compounds bearing an electrophilic center. These enzymes facilitate the nucleophilic attack of the -SH group of reduced glutathione (GSH) on the electrophilic centers of appropriate substrates which is the first step in the physiological formation of mercapturic acids.

In mammals, GSH-Tr activity has been demonstrated in the cytosolic and, in some cases, microsomal fractions of the cells of various organs. In the liver, these enzymes constitute from 3-10% of the cytosolic proteins. With the possible exception of the erythrocyte, GSH-Tr exists in cells in multiple molecular forms. It has been reported that the isozymes GSH-Tr can be distinguished from one another by their substrate specificities.

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Metal-Induced Inhibition of Glutathione S-Transferases

The glutathione S-transferases comprise a group of multi-functional enzymes involved in the biotransformation/detoxication of a broad spectrum of hydrophobic compounds bearing an electrophilic center. These enzymes facilitate the nucleophilic attack of the -SH group of reduced glutathione (GSH) on the electrophilic centers of appropriate substrates which is the first step in the physiological formation of mercapturic acids.

In mammals, GSH-Tr activity has been demonstrated in the cytosolic and, in some cases, microsomal fractions of the cells of various organs. In the liver, these enzymes constitute from 3-10% of the cytosolic proteins. With the possible exception of the erythrocyte, GSH-Tr exists in cells in multiple molecular forms. It has been reported that the isozymes GSH-Tr can be distinguished from one another by their substrate specificities.

In addition to their multitude of catalytic functions, the GSH-Trs, especially GSH-TrB or ligandin, bind, nonenzymatically, a number of nonsubstrate ligands such as bilirubin, steroids and steroid metabolites, and a broad spectrum of toxic compounds.

Certain GSH-Trs also express glutathione peroxidase activity which is directed only toward organic hydroperoxides. Unlike the GSH peroxidase activity which is selenium-dependent, this activity is selenium independent.

THE ENZYME INVOLVED HAS BEEN TERMED NON-SELENIUM GSH PEROXIDASE OR GSH PEROXIDASE II (GSH-PxII) TO DIFFERENTIATE IT FROM THE SE-DEPENDENT ENZYME WHICH IS ACTIVE TOWARD BOTH HYDROGEN PEROXIDE AND ORGANIC HYDROPEROXIDES. THE GSH PEROXIDASE CATALYTIC FUNCTION OF GSH-TRS HAS BEEN IMPLICATED IN THE PROTECTION OF CELLULAR MEMBRANES FROM DAMAGE DUE TO LIPID PEROXIDATION.

IN TECHNOLOGICAL SOCIETIES, MAN IS CONTINUOUSLY EXPOSED TO A VARIETY OF HAZARDOUS CHEMICALS, INCLUDING TOXIC METALS SUCH AS CADMIUM, MERCURY, AND LEAD, ETC. THERE IS CONSIDERABLE SPECULATION AND CONTROVERSY REGARDING THE MODE OF TOXIC ACTION OF METALS. ONE MECHANISM SUGGESTED IS THAT THE TOXICITY OF SOME HEAVY METALS MAY BE MEDIATED THROUGH PEROXIDATION OF MEMBRANE LIPIDS. THIS IS CONSISTENT WITH THE HYPOTHESIS THAT ORGANOMETALLIC COMPOUNDS IN GENERAL AND METALS SUCH AS MERCURY, SILVER, CADMIUM, AND LEAD THAT UNDERGO UNIVALENT REDOX REACTIONS MAY PROMOTE TISSUE DAMAGE THROUGH: RADICAL-INDUCED MECHANISMS OR IRREVERSIBLE INHIBITION OF ENZYMES INVOLVED IN ANTIOXIDANT DEFENSE FUNCTIONS SUCH AS GSH PEROXIDASE AND SUPEROXIDE DISMUTASE. INDEED, IT HAS BEEN DEMONSTRATED THAT RAT LIVER SELENIUM-DEPENDENT GSH PEROXIDASE IS INHIBITED BY CADMIUM, ZINC, SILVER, AND OTHER METAL IONS. HOWEVER, METAL-INDUCED INHIBITION OF GSH-TRS HAS NOT BEEN EXTENSIVELY INVESTIGATED AND INHIBITION OF GSH PX II ACTIVITY, WHICH APPEARS TO HAVE IMPORTANT ANTIOXIDANT DEFENSE FUNCTIONS, COULD HAVE PROFOUND CONSEQUENCES, ESPECIALLY UNDER CONDITIONS OF OXIDANT STRESS.

In addition, information on the molecular target(s) and mode of action of toxic metals is far from complete. Accordingly, I will review some of the information on the effects of metal ions, some of which have profound environmental importance, on the transferase and peroxidase activities of GSH-Tr from the point of view that the toxicity of such metal ions, at least in part, may be due to their interference with these enzymatic activities. As the point of departure, I will utilize data gathered in our laboratory.

In our study, GSH-Trs were purified from calf liver to approximately 70% of purity by a modification of the procedure of Habig et al. (1974A).

Slide #1

Transferase activity was assayed by monitoring the formation of the thioether between GSH and 1-chloro-2,4-dinitrobenzene (CDNB) by a modification of the procedure of Habig et al. (1974A).

Glutathione peroxidase activity was measured by monitoring the oxidation of NADPH at 340 nm by a modification of the procedure described by Paglia and Valentine (1967) with cumene hydroperoxide as substrate.

Since the GSH-Px assay method employs a coupled enzyme system involving GSH reductase, it is possible that inhibition of GSH-Px activity by metal ions or other agents, in part, may be due to inhibition of GSH reductase. Therefore, the direct spectrophotometric method of Splittgerber and Tappel (1979) also was employed to assay GSH-Px activity.

One unit of GSH-Tr activity is defined as the amount of enzyme catalyzing the formation of 1 μmol of thioether per minute at 25°C. One unit of GSH-Px activity is defined as the amount of enzyme catalyzing the oxidation of 1 μmol of NADPH per minute at 25°C (in the coupled assay system) or as a change of 1 ARS per minute at 25°C (in the direct spectrophotometric assay).

The protein concentration was determined by the method of Lowrey et al. (1951) with bovine serum albumin as the standard.

Inhibition of transferase or peroxidase activity by divalent metal ions was investigated by pre-incubating the enzyme in buffer with the metal salt of interest for 10 min prior to the addition of the other components of the reaction mixture. For all kinetic studies of inhibition, the initial reaction velocity was determined in the presence of varying concentrations of one substrate while the concentration of the other substrate was held constant. Identical determinations were made in the presence of fixed concentrations of the inhibitory metal ions. Data were analyzed by double reciprocal plots and X and Y intercepts were calculated by least-square regressions.

THE INHIBITORY KINETICS WERE ANALYZED BY THE METHOD OF DIXON (1953).

THE ENZYME WE INVESTIGATED HAD NO PEROXIDASE ACTIVITY WITH H_2O_2 AS SUBSTRATE.

SLIDE #2

AS SHOWN ON THIS SLIDE THAT, IN THE COUPLED ASSAY SYSTEM, TRANSFERASE ACTIVITY WAS MARKEDLY INHIBITED BY CADMIUM [Cd(II)], METHYLMERCURY, MERCURY [Hg(II)], AND LEAD [Pb(II)]. IT IS TO BE NOTED THAT A 50% INHIBITION WAS OBSERVED AT METAL ION CONCENTRATIONS AS LOW AS 10^{-5}M. OTHER METAL IONS SUCH AS COPPER, ZINC, AND MANGANESE WERE CONSIDERABLY LESS INHIBITORY OF THE TRANSFERASE ACTIVITY.

IT MUST BE RE-EMPHASIZED THAT, IN THE COUPLED ASSAY SYSTEM, IT IS POSSIBLE THAT THE OBSERVED METAL ION INHIBITION OF PEROXIDASE ACTIVITY COULD BE A REFLECTION, AT LEAST IN PART, OF INHIBITION OF GLUTATHIONE REDUCTASE, THE ENZYME INVOLVED IN THE CONVERSION OF OXIDIZED GLUTATHIONE (GSSG) TO GSH UTILIZING NADPH AS THE ELECTRON DONOR. HOWEVER, INCLUSION OF A LARGE EXCESS OF GLUTATHIONE REDUCTASE (UP TO 50 IU) IN THE REACTION MIXTURE HAD NO EFFECT ON THE PATTERN OF INHIBITION, WHICH INDICATES THAT THE INHIBITION OBSERVED MAINLY WAS DUE TO THE EFFECT OF THE DIVALENT METAL IONS ON THE PEROXIDASE ACTIVITY.

SLIDE #3

THIS WAS SUBSTANTIATED BY THE RESULTS OBTAINED WHEN INHIBITION WAS MONITORED BY THE DIRECT SPECTROPHOTOMETRIC METHOD OF SPLITTGERBER AND TAPPEL (1979), AS SHOWN ON THIS SLIDE.

IT IS EVIDENT THAT, AS IN THE CASE OF THE TRANSFERASE ACTIVITY, CD (II), PB (II), METHYLMERCURY, AND HG(II), MARKEDLY INHIBITED THE PEROXIDASE ACTIVITY. IN FACT, THE PEROXIDASE ACTIVITY WAS MORE SENSITIVE TO INHIBITION BY THESE METAL IONS THAN THE TRANSFERASE ACTIVITY AND WAS INHIBITED BY 50% AT CONCENTRATIONS AS LOW AS 10-6 M.

SLIDE #4

THE NATURE OF THE INHIBITION OF GSH-TR ACTIVITY BY METAL IONS WAS INVESTIGATED EMPLOYING FIXED CONCENTRATIONS OF CD(II), METHYLMERCURY, HG(II), AND PB(II) AND VARYING CONCENTRATIONS EITHER OF GSH OR CDNB. THIS SLIDE SHOWS THE DOUBLE-RECIPROCAL PLOTS OF INHIBITION OF THE TRANSFERASE ACTIVITY BY THE METAL IONS. THE CONCENTRATION OF GSH WAS VARIED FROM 0.025 TO 2.5 MM WHILE THE CONCENTRATION OF CDNB WAS MAINTAINED AT 1 MM. IT WOULD APPEAR THAT INHIBITION OF TRANSFERASE ACTIVITY AT VARYING CONCENTRATIONS OF GSH IS NONCOMPETITIVE.

SLIDE #5

AS CAN BE SEEN, WITH VARYNG CONCENTRATIONS OF CDNB, NONCOMPETITIVE INHIBITION ALSO WAS OBSERVED.

SLIDE #5A

THE TRANSFERASE INHIBITION CONSTANTS (K_I) OF THE METAL IONS WERE CALCULATED FROM PLOTS OF INTERCEPTS VERSUS INHIBITOR CONCENTRATIONS AND THE K_I VALUES OBTAINED WERE 2, 4, 6.5 AND 9 x 10^{-5} M FOR CD(II), METHYLMERCURY, HG(II), AND PB(II), RESPECTIVELY.

SLIDE #6

WHEN GSH-PX II ACTIVITY WAS MEASURED WITH CONCENTRATIONS OF GSH RANGING FROM 0.025 TO 2.5 mM , THE VELOCITY VS GSH CONCENTRATION PLOT WAS BIPHASIC AND THE K_M VALUES FOR GSH WERE CALCULATED TO BE 4 x 10^{-5} AND 1.2 x 10^{-4}M. ALSO, WHEN THE PEROXIDASE ACTIVITY WAS MEASURED AT FIXED CONCENTRATIONS OF CD(II) AND VARYING CONCENTRATIONS OF GSH BETWEEN 0.025 AND 2.5 mM, THE PATTERN OF DOUBLE RECIPROCAL PLOTS OBTAINED WAS COMPLICATED, REFLECTING A COMPLEX INTERACTION AMONG CD, THE ENZYME, AND GSH.

SLIDE #7

HOWEVER, AS SHOWN ON THIS SLIDE, IN THE RANGE 0.1 TO 2.5 mM GSH, WHERE THE VELOCITY VS GSH CONCENTRATION PLOT VS LINEAR, INHIBITION OF GSH-PX II ACTIVITY BY CD(II), METHYLMERCURY, HG(II), AND PB(II) IS NONCOMPETITIVE.

SLIDE #8

ALSO, AS SHOWN ON THIS SLIDE, AT FIXED CONCENTRATIONS OF CD(II), HG (II), METHYLMERCURY, OR LEAD (II) AND VARYING CONCENTRATIONS OF COP, NONCOMPETITIVE INHIBITION WAS OBSERVED.

SLIDE #8A

PLOTS OF INTERCEPTS VS INHIBITOR CONCENTRATIONS GAVE GSH-PX II K_I VALUES OF 1.5, 7, 9, AND 3 x 10^{-6}M, RESPECTIVELY, FOR CD(II), METHYLMERCURY, HG(II), AND PB(II).

SLIDE OFF,PLEASE

SEVERAL REDUCING AGENTS SUCH AS β-MERCAPTOETHANOL, DITHIOTHREITOL, AND CYSTEINE WERE TESTED IN PLACE OF GSH FOR THEIR ABILITY TO REVERSE THE METAL ION-INDUCED INHIBITION OF GSH-PX II. ALL WERE INEFFECTIVE.

SINCE CD(II), METHYLMERCURY, HG(II), AND PB(II) ARE WIDELY DISTRIBUTED ENVIRONMENTAL POLLUTANTS, IT IS OF IMPORTANCE TO GAIN UNDERSTANDING OF THEIR MOLECULAR TARGETS AND THE MECHANISM(S) BY WHICH THEY EXERT THEIR TOXIC EFFECTS. INDEED, MANY INVESTIGATORS HAVE DIRECTED CONSIDERABLE EFFORT TOWARD THE ACHIEVEMENT OF THESE GOALS. IN ADDITION, INTEREST IN THE MODE OF TOXIC ACTION OF OTHER METALS CONTINUES TO DEVELOP AND PROVIDE INFORMATION APPLICABLE TO EXISTING PROBLEMS. FOR EXAMPLE, INTEREST IN THE TOXICITY OF ORGANOTIN COMPOUNDS HAS, IN RECENT YEARS, INCREASED DRAMATICALLY. TRIALKYL- AND TRIARYLTIN COMPOUNDS ARE POTENT NEUROTOXINS AND CAUSE CENTRAL NERVOUS SYSTEM EDEMA IN HUMANS AND OTHER MAMMALS, DECREASED GLUCOSE METABOLISM BY THE BRAIN, AND INDUCE DEPLETION OF BRAIN AND CARDIAC CATECHOLAMINES. IN <u>IN VITRO</u> EXPERIMENTS, IT HAS BEEN OBSERVED THAT COMPOUNDS WITH THREE CARBON-TIN BONDS INHIBIT MITOCHONDRIAL OXIDATIVE PHOSPHORYLATION, AND ATPASE AND PYRUVATE KINASE ACTIVITY. THE MORE LIPID SOLUBLE ORGANOTINS ALSO EXHIBIT HEMOLYTIC ACTIVITY.

As we and others have demonstrated, Cd(II), methylmercury, Hg(II), and Pb(II) strongly inhibit both the transferase and peroxidase activities of GSH-Tr in a noncompetitive manner with respect to the substrates tested. Compounds having three carbon-tin, carbon-lead, or carbon-germanium bonds inhibit rat liver GSH S-aryltransferase activity in a competitive manner with 1,2-dichloro-4-nitrobenzene as the limiting substrate with K_I values in the μM range. Also, triethyltin (Et_3Sn) inhibits GSH S-Trs A,B, and C in a competitive manner with 1-chloro-2,4-dinitrobenzene as the limiting substrate.

The exact mechanism(s) by which Cd(II), methylmercury, Hg(II), and Pb(II) ions interact with GSH-Tr to bring about this inhibition is not clear. However, it is well known that divalent metal ions bind to -SH groups and, a priori, it would seem reasonable to assume that Cd(II), Hg(II), and Pb(II) might compete with the enzyme protein for GSH. In such cases metal ion inhibition should be reversed by the addition of excess GSH to the reaction mixture. This, in effect, is the definition of competitive inhibition.

As has been demonstrated, preincubation with submicromolar concentrations of Cd(II), methylmercury, Hg(II), and Pb(II) markedly inhibit both the GSH-Tr and GSH-Px activities of the enzyme. However, in our hands the inhibition could not be reversed by the subsequent addition of a several hundred-fold excess of GSH which suggests that the inhibition induced by these metals is not due to competition for GSH.

THIS IS SUPPORTED BY THE NONCOMPETITIVE NATURE OF THE INHIBITION WITH RESPECT TO GSH. MOREOVER, IN THAT THE BINDING SITE ON THE ENZYME FOR THE ORGANIC SUBSTRATES IS HYDROPHOBIC IN NATURE, IT WOULD BE EXPECTED TO HAVE A RELATIVELY LOW AFFINITY FOR METAL IONS. THEREFORE, IT SEEMS UNLIKELY THAT THE METAL IONS WOULD COMPETE FOR BINDING WITH THE ORGANIC SUBSTRATES CDNB OR COP. IN THE LIGHT OF THESE OBSERVATIONS, IT MAY BE SPECULATED THAT THE METAL IONS INHIBIT THE ENZYME BY INTERACTING WITH AN ESSENTIAL GROUP(S) ON THE PROTEIN WHICH PROMOTE THE CATALYTIC FUNCTION. THE NATURE OF THESE ESSENTIAL GROUPS IS, AT PRESENT, UNKNOWN. HOWEVER, IT HAS BEEN REPORTED BY DIERICKX (1982) THAT WHEN SUBSTRATE AND INHIBITOR ARE ADDED TO THE REACTION MIXTURE IN RAPID SUCCESSION, ATTENUATION OF THE INHIBITORY EFFECTS OF HG AND CU, BUT NOT CD, WAS OBSERVED AT INCREASING CONCENTRATIONS OF GSH, CYSTEAMINE, CYSTEINE AND PROTEIN. BUT IT MUST BE NOTED THAT DIERICKX INVESTIGATED THE INHIBITION OF GSH-TR ACTIVITY IN THE CRUDE SUPERNATANT OBTAINED FROM RAT LIVER. LIKEWISE, HENRY AND BYINGTON (1975) HAVE REPORTED THAT BAL AND SODIUM SULFIDE PROTECT GSH S-ARYLTRANSFERASE FROM INHIBITION BY TRIPHENYL TIN (CHLORIDE). ALSO, THEY OBSERVED THAT BIS TRIPHENYLTIN SULFIDE WAS A WEAK INHIBITOR OF THE ENZYME AND SUGGESTED THAT THE PROTECTIVE EFFECT OF SULFUR COMPOUNDS MAY BE MEDIATED THROUGH THE FORMULATION OF DERIVATIVES OF THE INHIBITOR HAVING SULFUR-TIN BONDS. GEL EXCLUSION CHROMATOGRAPHY INDICATES THAT Et_3Sn AND GSH INTERACT IN AQUEOUS SOLUTION AND TIPPING ET AL. (1979) HAVE SUGGESTED THAT INHIBITION IS THE RESULT OF TERNARY COMPLEX FORMATION INVOLVING THE ENZYME, GSH, AND Et_3Sn, IN WHICH GSH AND Et_3Sn MAY OR MAY NOT INTERACT DIRECTLY.

Alternatively, inhibition may be the result of quaternary complex formation involving enzyme, GSH, and Et_3S_N in a 1 to 2 to 1 stoichiometry. In any case, Tipping et al. (1979) reported that in equilibrium dialysis experiments carried out at 4°C, interaction of Et_3S_N with transferases A and B was weak: the KD being essentially $7.0\ 10^{-3}$ M (7.1×10^{-3} M) and 3.0×10^{-4} M (3.4×10^{-4} M) for GSH-Trs A and B, respectively, compared to 2.0×10^{-7} M and approximately 3.0×10^{-5} M (2.6×10^{-5} M) for GSH-Trs A and B, respectively, in the presence of 5.0×10^{-3} M GSH. Although the enzyme preparations used in these studies apparently contained more than one form of GSH-Tr, the linearity of the double reciprocal plots suggests that the isozymes exhibit similar binding kinetics. Also, we have observed a similar binding kinetics with other isozymes of GSH-Tr which indicates that the metal ions may bind to a functional group common to all isozymes of GSH-Tr.

It has been suggested that the toxicity of methylmercury, Hg(II), Cd(II), and Pb(II) is mediated via the promotion of lipid peroxidation. Since GSH-Px II appears to be an important component of the antioxidant defense mechanism of various tissues, it is tempting to suggest that the toxicity of such metal ions may be due to their strong inhibitory effects on GSH-Px II. Recently, Black and co-workers (1979) reported inhibition of the GSH-Px activity (assayed both with H_2O_2 and COP) of various tissues of rats exposed to Hg(II) and Ag(I). However they did not find any significant reduction of GSH peroxidase activity in rats exposed to either Cd(II) or Pb(II).

FINDING OF A STRONG INHIBITORY EFFECT OF Cd(II) AND Pb(II) ON GSH-Px II ACTIVITY AND THAT OF OMAYE AND TAPPEL (1975) WHO OBSERVED THAT Cd(II)-INDUCED TESTICULAR DAMAGE IN RATS WAS ASSOCIATED WITH INHIBITION OF GSH-Px ACTIVITY.

WHILE, THE HYPOTHESIS THAT THE TOXICITY OF METAL IONS MAY BE MEDIATED THROUGH THE INHIBITION OF KEY ANTIOXIDANT DEFENSE ENZYMES SUCH AS SELENIUM DEPENDENT GSH-Px, GSH-Px II, ETC. IS ATTRACTIVE, THE ACTUAL MODE OF TOXIC ACTION OF THESE METALS IS LESS THAN CERTAIN.

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